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Proteomic Analysis by Two-Dimensional Gel Electrophoresis and Starch Characterization of *Triticum turgidum* L. var. *durum* Cultivars for Pasta Making

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Criteria for durum wheat quality are continuously evolving in response to market pressure and consumer's preference. Specific attributes of durum wheat for different end products require more rapid and objective means to grade and classify wheat parcels based on processing potential. A total of 10 durum wheat cultivars were compared for compositional, protein, and starch characteristics. Mean values for the gross composition differed for total protein, gluten, and starch. Two-dimensional electrophoresis (2DE) analysis showed the proteome diversity among the cultivars. As shown by the principal component analysis (PCA) applied to 2DE data of gliadin and glutenin fractions, cultivars differed mainly from the number of proteins and levels of protein expression. As determined by the rapid viscoanalyzer (RVA), swelling power, starch damage, amylose content, and starch pasting properties of 10 cultivars differed significantly. 2DE fingerprinting and amylose content seemed to distinguish specific cultivars being useful tools for selecting suitable durum wheat cultivars for pasta making.

KEYWORDS: ***Triticum durum*; proteome; starch; pasta; two-dimensional electrophoresis; rapid viscoanalyzer**

INTRODUCTION

Durum wheat (*Triticum turgidum* L. var. *durum*) is an important food crop in the Mediterranean area, not only because of its large acreage but also for its importance in the human diet (*1*). The annual world durum wheat production, over a 4 year period (2001−2004) has been estimated to be 36.3 million tons (*2*; INEA). Italy is one of the main producers of durum wheat, with ca. 4.0 million tons per year, exporting durum wheat to the world market (INEA). The major use of durum wheat is for pasta making, especially in the European and North American countries, whereas in other areas, it is also used for making bread, burghul, couscus, and frekeh. Currently, dietary recommendations from the U.S. Department of Agriculture−Health and Human Services (USDA−HHS) promote the consumption of pasta as an optimal source of complex carbohydrates, carotenoids, and low lipoxygenase activity (*3*). Pasta made from durum wheat semolina cultivars of superior quality results in a bright yellow color, retains firmness after cooking, and withstands surface disintegration and stickiness. Nevertheless, not all durum wheat semolina cultivars produce pasta of good cooking quality. Many variables are involved in pasta manufacturing, and their role is not completely understood (*4*; www.grainscanada.gc.ca/PUBS/confpaper/Dexter/trends/qualreq1-e.htm, Canadian Grain Commission).

The suitability of durum wheat cultivars for pasta making is mainly determined by the characteristics of seed proteins and starch (*5*). Gluten proteins consist of monomeric gliadins and polymeric glutenins. Gliadins are separated into four groups; α-, *β*-, *γ*-, and *ω*-gliadins. Gliadins are coded by the complex loci *Gli-1* (*γ* and *ω*) and *Gli-2* (α and *β*), located at the short arm of homologous chromosomes 1 and 6, respectively. Glutenins consist of high-molecular-weight (HMW-GS; 90−120 kDa) and low-molecular-weight (LMW-GS; 30−70 kDa) subunits linked together by disulphide bridges (*6*). On the basis of sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) mobility, three groups of LMW-GS have been identified: B-(MW 42−51

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kDa), C- (MW 30−40 kDa), and D-LMW-GS (MW 55−70 kDa) (*7*). HMW-GS are coded by complex loci *Glu-1*, located at the long arm of homologous chromosomes 1, whereas most of the LMW-GS are coded by complex loci *Glu-3*, closely linked to *Gli-1*. Separate loci coding for some intermediate and fast moving B subunits are present on chromosome 1B. According to the allelic forms at *Gli-B1*/*Glu-B3* loci, durum wheat cultivars are usually grouped into two main types. One type includes γ-gliadin 42 (γ-42) and the associated (1 cM recombination) LMW-GS type 1 (LMW-1); the other includes γ-gliadin 45 (γ-45) and the LMW-GS type 2 (LMW-2).

Glutenin polymers are responsible for the unique viscoelastic properties of wheat flour dough, while α-, β-, γ-, and ω-gliadins contribute to dough extensibility. Pasta quality and cooking characteristics are dependent upon the protein−starch matrix of the extruded pasta product (*8*). Characteristics such as firmness, cooking loss, and stickiness of pasta may be due to the concentration of proteins (*4, 9*) and gluten strength (*10*), as well as to the starch composition (*11, 12*). Currently, a number of assays are used for predicting the cooking quality of durum wheat pasta, e.g., concentration of proteins and gluten, protein composition (*13*−*15*), mixograph mixing, alveograph characteristics (*16*), sedimentation volume (*17*), swelling index of glutenin (*18, 19*), pigment, gluten thermal stability (thereafter, cooked gluten viscoelasticity) (*20*), pasta disk viscoelasticity (*21*), and sensory tests (*16*).

However, globalization and increasing competition in pasta industries made it indispensable to preliminarily define the cooking quality of durum wheat cultivars. Under these circumstances, industries need to set up efficient and discriminating analytical methods for evaluating the quality of proteins and starch semolina before subsequent processing (www.grainscanada.gc.ca/PUBS/confpaper/Dexter/trends/qualreq1-e.htm, Canadian Grain Commission).

This study describes the compositional, protein, and starch characteristics of 10 durum wheat cultivars by using an integrate approach of traditional and modern analytical techniques.

MATERIALS AND METHODS

Durum Wheat Samples and Milling. Eight Italian durum wheat cultivars (Arcangelo, Ciccio, Claudio, Colosseo, Duilio, Gargano, Simeto, and Svevo) were used. Samples were supplied by Divella SpA (Rutigliano, Bari, Italy). In the year 2005, cultivars were grown at the same location of the Apulia region (Southern Italy) and under the same agro-ecological conditions. Two other cultivars were also considered: Ocotillo from Arizona and Wollaroi from Queensland, Australia.

Grain was hand-cleaned, conditioned to 15% moisture overnight, and milled into semolina with a yield of ca. 55% under industrial conditions by Divella S.p.A.

Compositional Analysis. The particle size of semolina was determined according to Italian legislation methods (DPR 09.02.2001). Moisture and ash values were determined by the approved American Association of Cereal Chemists (AACC) methods (*22*). Protein analysis was carried out by the Kjeldhal method and expressed using the conversion factor $N \times 5.7$ (*23*). All determinations were carried out on three different samples of each semolina, and values were averaged.

Determination of Gluten and Swelling Index of Glutenin. Gluten was extracted using water or 2% NaCl solution, and the determination of the viscoelasticity, as relative recovery (Rr %, using 1.5 kg load weight), was carried out as described by Kovacs et al. (*20*) with some modifications. A total of 15 g of flour were assayed to get more gluten, and six gluten disks were used. Cooking time lasted 2 min. Cooked gluten density was determined by weighing the uniformly stamped out cooked gluten discs (*20*).

The swelling index of glutenin (SIG) was determined as described by Wang and Kovacs (*18, 19*).

Protein Extraction for Two-Dimensional Electrophoresis (2DE). Protein fractions were extracted from semolina by the method originally described by Weiss et al. (*24*). Briefly, an aliquot of semolina (12.75 g) was diluted with 30 mL of 50 mM Tris-HCl (pH 8.8), held at 4 °C for 1 h with vortexing at 15 min intervals, and centrifuged at 20000*g* for 20 min. The supernatant contained albumins and globulins. To minimize cross-contamination among albumins, globulins, and prolamins, pellets were further extracted twice with the same buffer and supernatants were discarded. After being washed with distilled water to remove buffer ions, pellets were suspended in 30 mL of ethanol (75%, v/v) and the suspension was stirred at 25 °C for 2 h and centrifuged as described above. The supernatant contained prolamins. The extraction by ethanol was repeated twice also. Residual ethanol was eliminated by suspending the pellets with distilled water and centrifugation. Finally, pellets were suspended in 4 mL of a urea−dithiothreitol (DTT) mixture [6 M urea, 1% (v/v) Triton X-100, 0.5% (w/v) DTT, and 0.5% (v/v) 2D Pharmalite at pH 3−10], held for 2 h at room temperature with occasional vortexing, and then centrifuged. The supernatant contained glutelins. The concentration of proteins of each fraction was determined by the Bradford method (*25*).

2DE. 2DE was performed with the immobiline−polyacrilamide system as described by Bjellqvist et al. (*26*) and Di Cagno et al. (*27*). Aliquots of 30 μg of semolina proteins were used for the electrophoretic run. Isoelectric focusing (IEF) was carried out on immobiline strips, providing a non-linear pH gradient from 3.0 to 10.0 and from 6 to 11 (IPG strips; GE Healthcare Bio-Sciences AB, Uppsala, Sweden) by IPG-phore, at 20 °C. The voltages were the following: 0−300 V for 1 h, 300−500 V for 3 h, 500−2000 V for 4 h, and a constant 8000 V for 4 h. After electrophoresis, IPG strips were equilibrated for 12 min against buffer A [6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, 0.05 M Tris-HCl (pH 6.8), and 2% (w/v) DTT] and for 5 min against buffer B [6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, 0.05 M Tris-HCl (pH 6.8), 2.5% (w/v) iodoacetamide, and 0.5% bromophenol blue].

The second dimension was carried out in a Laemmli system (*28*) on 13.5% polyacrilamide gels (13 cm × 20 cm × 1.5 mm) at a constant current of 40 mA/gel and at 15 °C for approximately 5 h, until the dye front reached the bottom of the gel. Gels were calibrated through: comigration of the extracts with human serum proteins for a molecular mass range from 200 to 10 kDa. Electrophoretic coordinates used for serum were described by Bjellqvist et al. (*26*). Gels were silver-stained as described by Hochstrasser et al. (*29*).

Multivariate Analysis of 2DE Protein Patterns. Protein maps were scanned with the Image Scanner and analyzed with Image Master 2D version 3.01 computer software (GE Healthcare Bio-Sciences AB). Principal component analysis (PCA) was carried out as described by Jacobsen et al. (*30*). Three gels were analyzed for each durum wheat semolina cultivar, and background subtraction and spot detection were performed automatically. The spot quantities were normalized by dividing the volume of each spot by the total volume over the whole image. In this way, differences in the color intensities between the gels were eliminated (*31*). Protein profiles and volume of each spots were exported from 2DE gels and used as variables for PCA analysis. The variable selection was performed by a cross-model validation (CMV) method, using the indicator variables as the *y* matrix and the protein spot volumes as the *x* matrix. CMV is an extended PLS/jack knife cross-validation method, but with an additional validation step. Each subset of samples is left out and validated against a model generated on the basis of cross-validation of the rest of the samples. Significance was reported as the percentage where each variable was marked as significant when tested on the external samples not included in the variable selection process. A cut off of 75% was chosen for the preliminary results to decrease the risk of missing some potential important proteins (*30*).

Determination of Total Starch and Amylose. The total concentration of starch was determined using the Megazyme starch assay kit (Megazyme Int., Wicklow, Ireland) method 76-13 (*22*). The concentration of amylose was determined as described by Gibson et al. (*32*) using the Megazyme amylose/amylopectin ratio assay kit.

Starch Damage. Starch damage in semolina was determined according to the Megazyme AACC method (*23*) using the kit Megazyme Starch Damage (Megazyme Int.). Samples of semolina (100

Table 1. Mean Values[a] for Moisture, Ash and Protein Characteristics of 10 Semolina Durum Wheat Cultivars

cultivar	moisture (%, wt/wt)	ash (%, dm[b])	protein (%, dm)	gluten (%, dm)	SIG[c] (g/g)	gliadin/glutenin
Arcangelo	14.00 ± 0.02 a	0.88 ± 0 d	13.00 ± 0.00 c	11.70 ± 0.11 c	3.10 ± 0.15 a	1.52 ± 0.07 d
Ciccio	14.10 ± 0.20 a	0.84 ± 0 b	11.60 ± 0.06 ab	10.30 ± 0.01 a	5.90 ± 0.06 c	0.97 ± 0.01 b
Claudio	14.10 ± 0.04 a	0.87 ± 0.01 c	12.10 ± 0.12 b	10.80 ± 0.01 b	3.90 ± 0.01 b	1.94 ± 0.04 f
Colosseo	14.50 ± 0.04 bc	0.84 ± 0 b	13.80 ± 0.05 d	12.40 ± 0.3 d	5.70 ± 0.07 c	1.14 ± 0.02 c
Duilio	15.00 ± 0.01 e	0.85 ± 0 b	12.40 ± 0.10 b	10.70 ± 0.03 b	6.00 ± 0.09 cd	0.55 ± 0 a
Gargano	14.80 ± 0.01 d	0.9 ± 0.01 c	13.00 ± 0.01 c	12.00 ± 0.13 cd	3.70 ± 0.22 ab	1.75 ± 0.03 e
Ocotillo	14.40 ± 0.04 b	0.8 ± 0 a	14.70 ± 0.04 e	13.30 ± 0.27 e	6.40 ± 0.03 e	0.99 ± 0.01 b
Simeto	14.60 ± 0.02 c	0.8 ± 0 a	11.70 ± 0.07 ab	10.20 ± 0.02 a	6.20 ± 0 d	1.00 ± 0.01 b
Svevo	14.90 ± 0.03 de	0.81 ± 0.01 a	11.50 ± 0.04 a	10.30 ± 0.04 a	6.20 ± 0 d	1.00 ± 0 b
Wollaroi	14.50 ± 0.03 bc	0.8 ± 0 a	14.80 ± 0.03 e	13.38 ± 0.26 e	6.50 ± 0.04 e	0.55 ± 0 a

[a] Data were expressed as the mean ± standard error of the mean. Means within each column labeled with different lowercase letters are significantly different according to the Tukey's test at $p \leq 0.05$. [b] Dry matter. [c] Swelling index of glutenin.

mg) were incubated with fungal α-amylase designed to give near complete solubilization of damaged granules with minimum breakdown of undamaged granules, and the reaction was stopped with the addition of diluted sulfuric acid. After centrifugation, the supernatant was treated with amyloglucosidase to give complete degradation of starch-derived dextrins to glucose. This was measured with the addition of the glucose oxidase peroxidase (GOPOD) reagent and used for calculating the starch damage as the percentage of weight of semolina. Damaged starch (7%) from maize was used as the standard.

Swelling Power (SP). SP was measured by a small-scale swelling test, according to the following procedure (*33*). Semolina (>33 mg) was weighed in a preweighed 2 mL Eppendorf tube, and 1 mL of water containing 0.5 mM of silver nitrate was added. The tube was capped, vortexed for 5 s, and placed in a thermomixer (Eppendorf, Germany) at constant temperature (95 ± 1 °C) and 1400 rev/min. After 30 min of heating and shaking, tubes were placed in a water bath at room temperature and then centrifuged (15000*g* for 10 min). The supernatant was carefully removed, and tubes containing gel were weighed. SP was calculated as the weight of sediment gel divided by the original weight of the sample.

Starch Paste Viscosity. Starch paste viscosity was measured by the rapid visco analyzer (RVA model Super 3, Newport Scientific, Sidney, Australia), according to Deffenbaugh and Walker (*34*) and the method ICC (*35*). Starch (3 g, 14% moisture) was mixed with 25 mL of 50 mM silver nitrate in a RVA canister. The suspension was heated from 50 to 95 °C at a rate of 12.2 °C/min, held at 95 °C for 2.5 min, and then cooled to 50 °C at a rate of 11.8 °C/min. The following variables were recorded in duplicate: peak viscosity (PV), breakdown (BK), trough (TG), final paste viscosity (FV), peak time (Pt), pasting temperature (PT), and setback (SB). Viscosity was expressed in centiPoise (cP). The reproducibility of the RVA method was determined by analyzing wheat starch 8 times at the same concentration.

Statistical Analysis. All data were obtained at least in triplicates. Percentages were arcsine-transformed for data analysis. Analysis of variance (ANOVA) was carried out on transformed data followed by the separation of means with Tukey's HSD using a statistical software Statistica for Windows (Statistica 6.0 per Windows 1998, StatSoft, Vigonza, Italia). Letters indicate significant different groups ($p < 0.05$) by Tukey's test. Multivariate statistical analysis (MSA) and PCA were carried out using Statistica 6.0 per Windows 1998 (StatSoft).

RESULTS

Compositional Analysis. All samples of durum wheat semolina had values of moisture ranging from 14.0 to 15.0% (wt/wt) (**Table 1**). The concentration of ash was rather uniform in all semolina samples and ranged from 0.80 to 0.90% (wt/wt). Protein and gluten ranged from 11.50−14.80 and 10.20−13.38% (wt/wt), respectively. The highest values (>12%) of total protein were according to the following order: Wollaroi > Ocotillo > Colosseo > Arcangelo and Gargano > Duilio > Claudio. The values found for protein were in agreement with those found for the concentration of gluten. The lowest values of gluten were found for Simeto, Svevo, and Ciccio (10.20−10.30%). The SIG analysis measures the swelling capacity of glutenins in wheat semolina and predicts the dough properties depending upon the level of insoluble glutenins (*18, 19*). The highest values of SIG were found for Wollaroi and Ocotillo (6.5 g/g), Simeto and Svevo (6.2 g/g), Duilio and Ciccio (ca. 6.0 g/g), and Colosseo (5.7 g/g). The other cultivars showed values in the range of 3.1−3.9 g/g. Large differences were found regarding the concentration of gliadin and glutenin fractions. The ratio of gliadin/glutenin ranged from 0.55 to 1.94 (**Table 1**).

Table 2. Mean Values[a] for Starch Characteristics of 10 Semolina Durum Wheat Cultivars

cultivar	total starch (%)	amilose (%)	damaged starch (%)	swelling power (g/g)
Arcangelo	65.7 ± 1.1 bc	13.5 ± 0.7 a	3.9 ± 0.1 f	9.7 ± 0.1 d
Ciccio	63.0 ± 0.6 b	23.6 ± 3.1 c	3.8 ± 0.1 f	7.9 ± 0.2 b
Claudio	68.1 ± 1.8 c	13.0 ± 0.8 a	3.4 ± 0.0 d	10.3 ± 0.5 d
Colosseo	73.2 ± 1.3 f	14.7 ± 0.3 b	3.1 ± 0.0 b	8.9 ± 0.0 c
Duilio	62.0 ± 0.8 ab	29.5 ± 1.0 c	3.7 ± 0.0 e	7.4 ± 0.1 a
Gargano	69.4 ± 1.9 d	16.3 ± 1.6 b	3.3 ± 0.0 c	8.8 ± 0.2 c
Ocotillo	61.0 ± 0.1 a	33.6 ± 0.7 d	3.1 ± 0.1 b	6.9 ± 0.2 a
Simeto	61.0 ± 0.5 a	33.1 ± 1.7 d	3.9 ± 0.1 f	7.4 ± 0.1 a
Svevo	70.7 ± 4.3 e	26.2 ± 1.3 c	3.3 ± 0.0 c	8.5 ± 0.5 c
Wollaroi	61.0 ± 0.5 a	26.9 ± 1.1 c	2.8 ± 0.1 a	6.9 ± 0.1 a

[a] Data were expressed as the mean ± standard error of the mean. Means within each column labeled with different lowercase letters are significantly different according to the Tukey's test at $p \leq 0.05$.

2DE. Gliadin and glutenin fractions were separated and visualized in triplicate by high-resolution 2DE. The reproducibility of the 2DE gel performance was analyzed by comparing samples in triplicate to the other 2DE gels. The chemical properties of gliadin proteins were very similar. As shown by 2DE with 3−10 IPG strips, most of the extracted proteins had values of p*I* ranging from ca. 4.2 to 9.0 and the molecular weight (MW) varied from ca. 18 to 94 kDa. The reproducibility of 2DE patterns was in focus because the gliadin proteins are very complex mixtures, where some of the proteins run very close to each other and the concentrations of individual proteins were different. To resolve the closed proteins, an IEF with 6−11 IPG strips was carried out. A total of ca. 25 proteins were commonly identified by 2DE analysis for all cultivars, and 2DE clearly resolved many individual α-, β-, γ-, and ω-gliadins. Overall, some differences were found for total spot numbers and expression of proteins between the 10 cultivars. The highest number of spot was found for Wollaroi (75 spots), Ocotillo (70 spots), Arcangelo (61 spots), Ciccio (59 spots), Colosseo (58 spots), and Gargano (51 spots). The slight contamination among protein fractions during selective extraction cannot be excluded (*30*). Overall, the highest protein expression was found for Ocotillo, Wollaroi, Ciccio, Colosseo, Claudio, Duilio, and

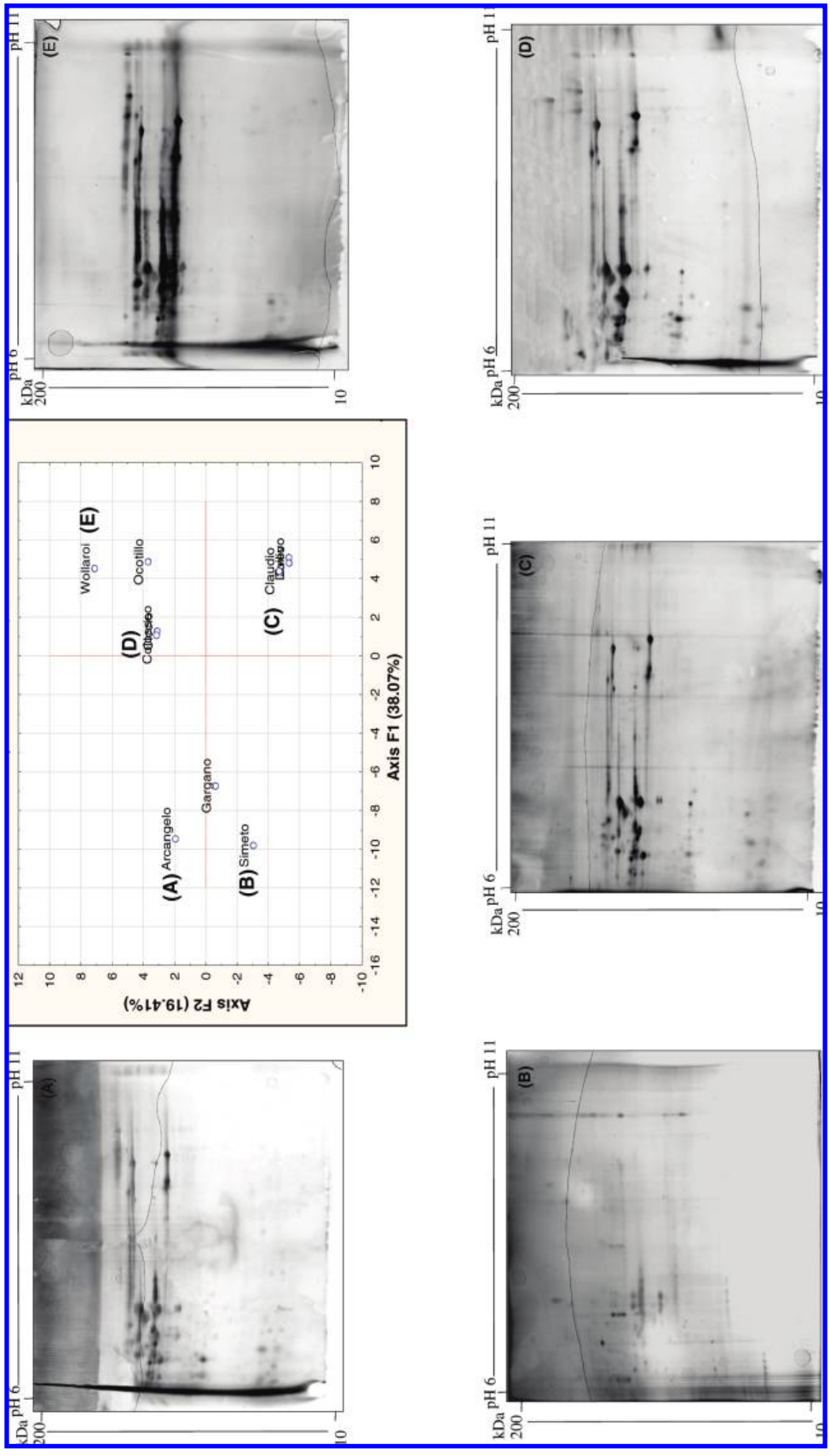

Figure 1. Score plot of the first and second principal components after PCA based on the data obtained from 2DE of gliadin proteins of 10 semolina durum wheat cultivars. Representative 2DE gels from (A) Arcangelo, (B) Simeto, (C) Claudio, (D) Colosseo, and (E) Wollaroi.

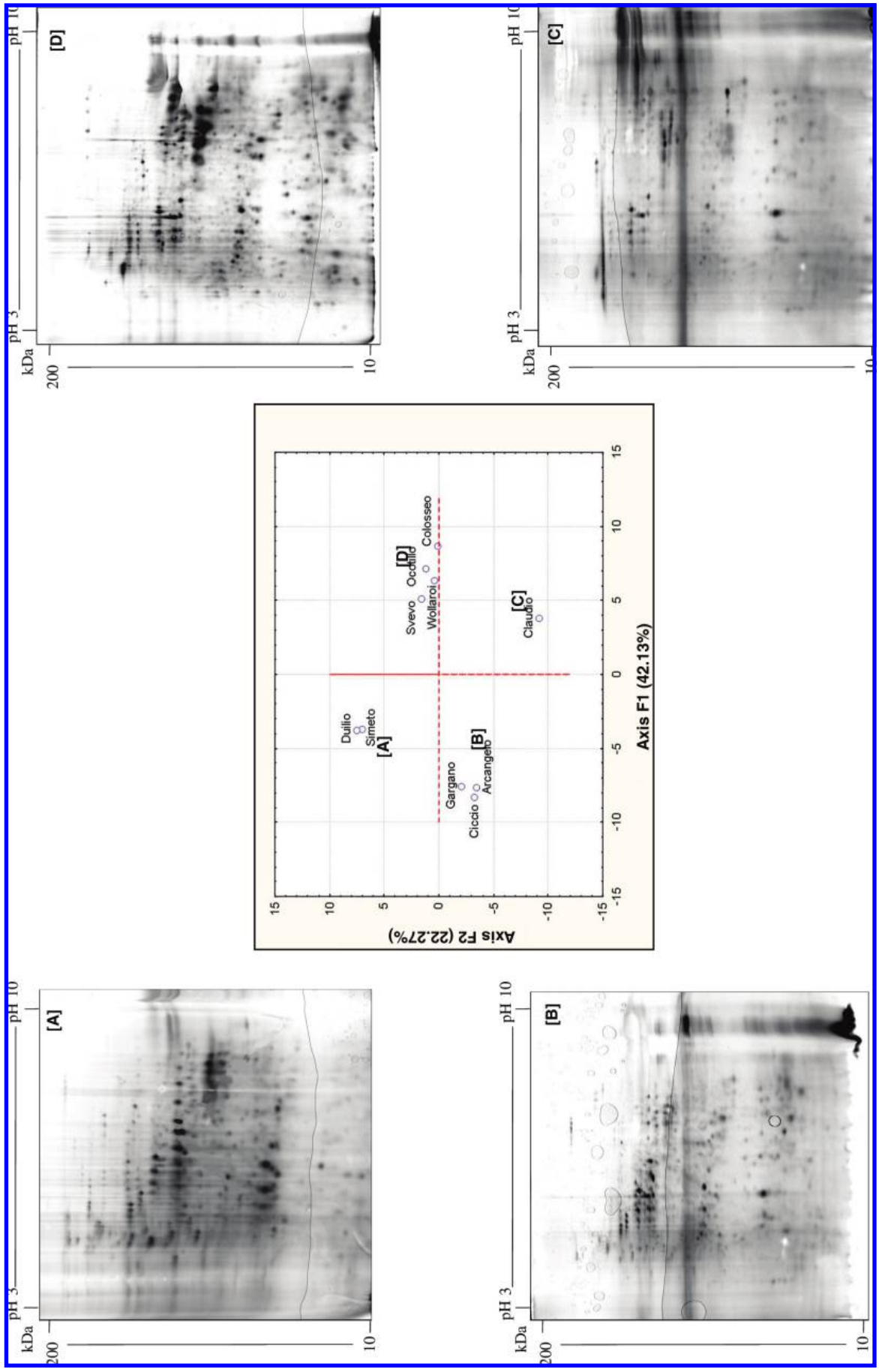

Figure 2. Score plot of the first and second principal components after PCA based on the data obtained from 2DE of glutenin proteins of 10 semolina durum wheat cultivars. Representative 2DE gels from (A) Simeto, (B) Arcangelo, (C) Claudio, and (D) Colosseo.

Table 3. RVA-Derived Starch Pasting Properties[a] (PT, Pasting Temperature; PV, Peak Viscosity; Pt, Peak Time; BK, Breakdown; TG, rough; SB, Setback; FV, Final Viscosity) of 10 Semolina Durum Wheat Cultivars

cultivar	PT (°C)	PV (cP)	Pt (min)	BK (cP)	TG (cP)	SB (cP)	FV (cP)
Arcangelo	59.0 ± 0.2 b	1885.5 ± 7.6 f	5.4 ± 0 a	430.0 ± 6.1 h	1455.5 ± 1.4 d	1512.5 ± 13.3 a	2968.0 ± 11.8 c
Ciccio	59.0 ± 0.2 b	1774.0 ± 0 e	5.5 ± 0 b	246.5 ± 1.0 e	1527.5 ± 1.0 e	1687.5 ± 0.6 d	3215.0 ± 1.6 e
Claudio	61.3 ± 0 d	2234.0 ± 5.3 i	5.6 ± 0 c	254.0 ± 5.3 e	1980.0 ± 0.2 i	1989.0 ± 5.3 e	3969.0 ± 5.3 h
Colosseo	60.3 ± 0.3 c	2043.5 ± 14.1 h	5.6 ± 0 c	211.0 ± 4.9 d	1832.5 ± 9.2 h	1977.5 ± 1.4 e	3810.0 ± 10.6 g
Duilio	58.6 ± 0 b	1295.5 ± 0.6 a	5.7 ± 0 d	22.5 ± 1.8 b	1273.0 ± 2.4 a	1600.0 ± 6.9 b	2873.0 ± 4.5 a
Gargano	59.0 ± 0.2 b	1445.5 ± 7.1 d	5.4 ± 0 a	326.0 ± 0.46 f	1587.5 ± 8.4 g	1672.0 ± 3.7 d	3259.5 ± 12.0 f
Ocotillo	60.4 ± 0 c	1314.5 ± 1.0 b	5.7 ± 0 d	0 a	1314.5 ± 1.0 b	1622.0 ± 3.7 c	2936.5 ± 2.7 b
Simeto	58.1 ± 0.2 a	1464.5 ± 7.6 d	5.5 ± 0 b	112.5 ± 3.1 c	1352.0 ± 4.5 c	1622.0 ± 4.5 c	2974.0 ± 0 d
Svevo	59.1 ± 0.2 b	1947.0 ± 4.9 g	5.6 ± 0 c	389.0 ± 2.0 g	1558.0 ± 2.9 f	1689.5 ± 5.5 d	3247.5 ± 8.4 f
Wollaroi	61.2 ± 0 d	1350.0 ± 1.2 c	5.6 ± 0 c	0 a	1350.0 ± 1.2 c	1601.5 ± 5.5 b	2951.5 ± 6.7 b

[a] Data were expressed as the mean ± standard error of the mean. Means within each column labeled with different lowercase letters are significantly different according to the Tukey's test at $p \leq 0.05$.

Svevo. On the basis of MW and p*I*, the ω-gliadin was the most abundant fraction for all of the cultivars (data not shown).

Among glutenins, a total of 41 proteins was commonly identified by 2DE analysis in all of the cultivars. They were widespread throughout MW from 94 to 15 kDa and p*I* from 3.8 to 8.9. The highest number of spots was found for Svevo (200 spots), Ocotillo (179 spots), Wollaroi (150 spots), Colosseo (109 spots), and Claudio, Arcangelo, and Simeto (ca. 97 spots). On the basis of MW and p*I*, the LMW-GS was the most abundant glutenin fraction for all of the cultivars.

PCA, a MSA technique, was used to reduce the number of dimension in the data set and visualize the structure of the data. This was achieved by transforming the observed variables into a new set of independent variables, termed independent variables (PCs). These were ordered in a way that the first PC explains most of the systematic variation of the data. Using the first few PCs only, a large part of the information data is shown in a simple 2D plot. On this basis, it was possible to identify outlying observations, clusters of similar observations, and other data structures (*36*). PCA was applied to the gliadin and glutenin 2DE patterns (**Figures 1** and **2**). Regarding gliadins, the two PCs (PC1 and PC2) explained ca. 57.4% of the total variance of the data (**Figure 1**). PC1 showed the level of expression of each proteins, and PC2 showed the total number of proteins. Simeto showed the lowest level of protein expression, and it was distributed in the negative quadrant defined by the two PCs. On the contrary, Wollaroi, Ocotillo, Colosseo, and Ciccio were distributed in the positive quadrant because they showed the highest level of protein expression and high numbers of spot. In comparison to Simeto, Claudio, Duilio, and Svevo were clustered together because they were characterized by the low number of spots with higher level of expression.

The PCA applied to glutenin 2DE patterns is shown in **Figure 2**. The PC1 and PC2 explained ca. 62.4% of the total variance of the data. The 10 cultivars were distributed in four different zones. Svevo, Ocotillo, Wollaroi, and Colosseo were clustered together because they showed the highest number of spots with a high level of protein expression. Gargano, Ciccio, and Arcangelo were located on the opposite zone of the plane, showing the lowest number of spots and having a low level of protein expression. Claudio was unclustered because it had a high level of HMW-GS and few LMW-GS proteins. On the contrary, Duilio and Simeto showed low levels of HMW-GS and high levels of LMW-GS and were located in the opposite zone of the plane.

Starch Characterization. The amylose/amylopectin ratio and total starch were determined using Megazyme methods. Significant variation in total starch ($p < 0.05$) between semolina cultivars was found (**Table 2**). Values of total starch ranged from 61.0 to 73.2%. In particular, Wollaroi, Ocotillo, Simeto, and Duilio cultivars were characterized by the lowest values (61.0−62.0%), while the highest concentration of starch was found for Colosseo > Svevo > Gargano.

The concentration of amylose of the 10 cultivars ranged from 13.0 to 33.6%. The lowest values of amylose (<20%) were found for Gargano > Colosseo > Arcangelo and Claudio, while the highest values (>30%) were found for Simeto and Ocotillo (**Table 2**). No statistical correlation was found between concentrations of total starch and amylose (data not shown).

During milling, some starch granules are mechanically damaged. Starch damage was determined by the Megazyme AACC method. The lowest values of damaged starch were found for Wollaroi (2.8%), Ocotillo and Colosseo (3.1%), Svevo and Gargano (3.3%), and Claudio (3.4%) (**Table 2**).

The flour SP method is usually used to assess intercultivar differences for starch properties (*33*). A narrow range of SP was found between the 10 semolina cultivars (6.9−10.3 g/g) (**Table 2**).

Pasting properties of starch were studied using the RVA method (**Table 3**). RVA parameters were pasting temperature (PT), peak viscosity (PV), peak time (Pt), breakdown (BK), trough (TG), setback (SB), and final viscosity (FV). Values of PT ranged from 58.1 to 61.3 °C. Overall, similar starch pasting curves were found for Wollaroi, Ocotillo, and Duilio, having the same low values of PV and BK (data not shown). On the contrary, cultivars Claudio, Colosseo, and Svevo had the highest values of PV (**Table 3**). The significantly ($p < 0.05$) highest Pt was detected for Claudio, Colosseo, Duilio, Ocotillo, Svevo, and Wollaroi (5.6−5.7 min). Values of TG and PV were similar for Duilio, Ocotillo, and Wollaroi. Large differences between TG and PV were found for Arcangelo and Svevo. SB and FV were the highest for Claudio and Colosseo cultivars. PCA was applied to starch data also (parts **A** and **B** of **Figure 3**). The two PCs explained ca. 78% of the total variance of the data. PC1 was related to viscosity properties (RVA data), and PC2 was related to the percentage of damaged starch. Overall, statistically significant correlations were found for SB × TG ($r = 0.904$), TG × PV ($r = 0.862$), SP × PV ($r = 0.792$), SP × amylose ($r = -0.734$), damaged starch × PT ($r = -0.705$), and amylose × PV ($r = -0.639$) (data not shown). Duilio, Ocotillo, and Wollaroi cultivars were located in a defined zone of the plot that was characterized for the high concentration of amylose and the low values of damaged starch. On the contrary, Arcangelo, Claudio, and Gargano showed a low concentration of amylose and high values of PV and TG.

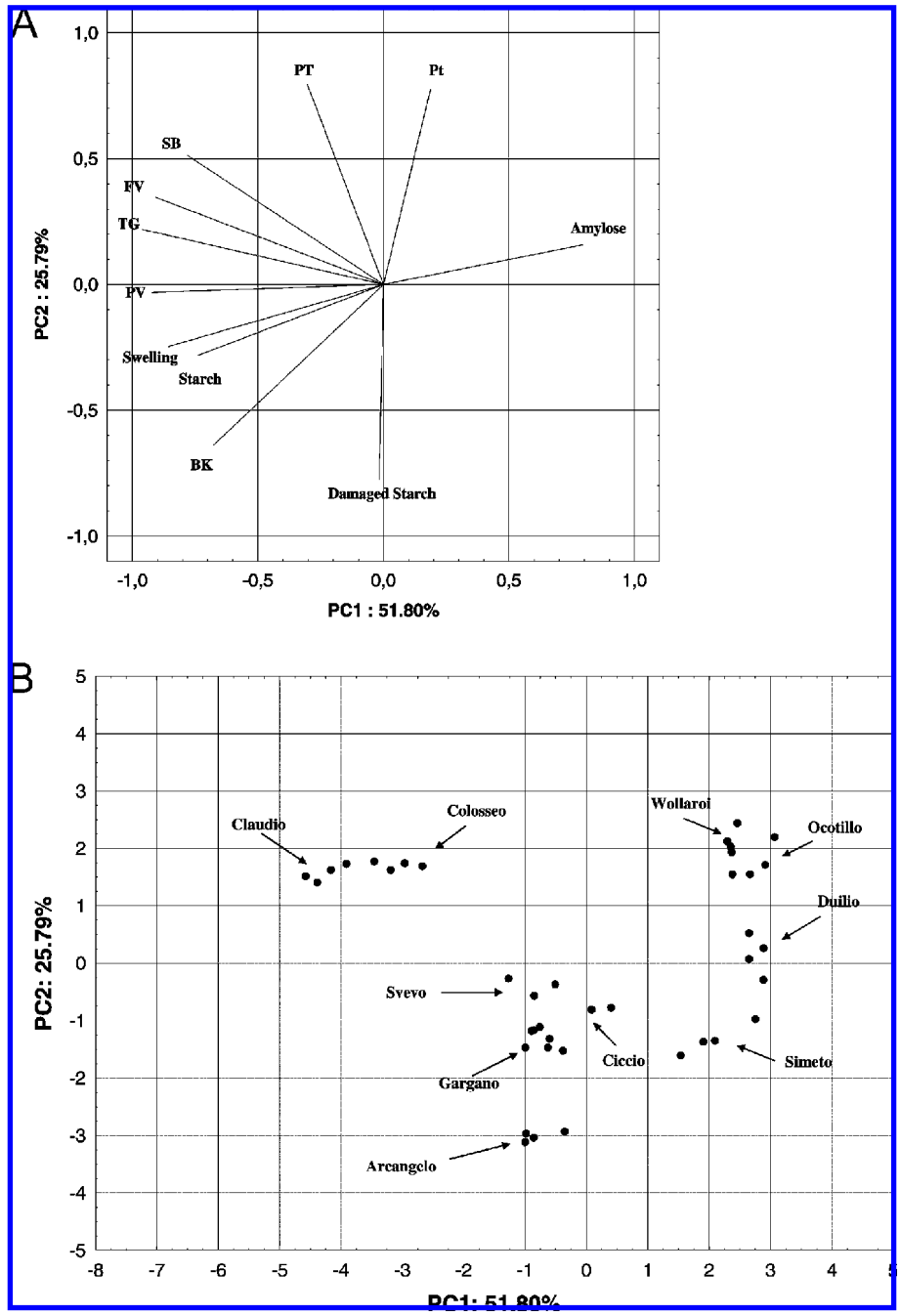

Figure 3. (A) Loading plot and (B) score plot of the first and second principal components after PCA based on the data obtained from starch analysis of 10 semolina durum wheat cultivars. Starch, total starch; amylose; damaged starch; Swelling, swelling power; PT, pasting temperature; PV, peak viscosity; Pt, peak time; BK, breakdown; TG, rough; SB, setback; FV, final viscosity.

DISCUSSION

Overall, good quality cooked pasta has to (i) maintain a good texture, (ii) be resistant to surface disintegration and stickiness, and (iii) retain firm structure or *al dente* consistency (*37*). Notwithstanding the role of processing, the above features are strictly related to durum wheat semolina quality. Currently, the concept of durum wheat quality could be considered as complex, evolving, and somewhat confusing. In particular, the effect of protein concentration in determining the pasta quality has been largely discussed (*38*). It has been clearly shown that the same concentration of protein may lead to contrasting rheology and cooking properties, thus indicating that characteristics other than gluten concentration are fundamental in pasta processing (*38*). This report used an integrate methodology approach to study the proteome and starch characteristics of 10 durum wheat semolina cultivars largely used for pasta making.

Except for moisture and ash, mean values for the gross composition were statistically different ($p < 0.05$) for cultivars. Semolina from Italian cultivars had the lowest values of total proteins and gluten, especially Svevo, Ciccio, and Simeto.

Five cultivars had values of SIG < 6.0 g/g. In particular, Arcangelo, Claudio, and Gargano cultivars showed very low concentrations of insoluble glutenins. Values of SIG were directly correlated to the SDS-insoluble HMW-GS and indirectly correlated to the cooking loss of pasta (*19*). According to values of SIG, the ratio of gliadin/glutenin was ≤1 for Ciccio, Duilio, Ocotillo, Simeto, Svevo, and Wollaroi, indicating good suitability of the semolina for cooking (*39, 40*).

Proteomic approaches using 2DE have provided new insights into protein composition of the endosperm as well as for processes involved in grain development, chromosomal locations of genes, and potential markers for genotype identification, and stress tolerance (*41*). Statistically significant ($p < 0.05$) differences were found within the proteome of the 10 cultivars. About 25 and 41 proteins belonging to gliadin and glutenin fractions, respectively, were commonly identified. Overall, the highest protein expression and/or number of proteins were found for Ocotillo, Wollaroi, Colosseo, Claudio, Duilio, and Svevo. On the basis of MW and p*I*, ω-gliadin and LMW-GS were the most abundant gliadin and glutenin fractions, respectively. Overall, studies that identified gluten strength as the prerequisite for getting pasta with a good texture have shown that lines expressing γ-gliadin-42 exhibited inferior pasta texture. On the contrary, cultivars expressing γ-gliadin-45 gave a cooking quality higher than the former (*42*), thanks to their strength rank, which varied from moderately to extraordinarily strong (*43, 44*). It was shown that pasta quality is associated with the presence of the specific allelic form of typical LMW-GS, named LMW-2 (*45*). These proteins are associated with dough resistance and extensibility, and some allelic forms of LMW-GS show even greater effects on these properties than HMW-GS. Nevertheless, the overexpression of HMW subunit trans-genes in tetraploid pasta wheat increased the dough strength and, therefore, the pasta quality (*46*). According to the allelic forms at *Gli-B1*/*Glu-B3* loci, durum wheat cultivars are usually grouped into two main types. One type includes γ-gliadin 42 (γ-42) and the associated (1 cM recombination) LMW-GS type 1 (LMW-1), and the other includes γ-gliadin 45 (γ-45) and the LMW-GS type 2 (LMW-2). The latter confers better properties to semolina (*38, 47*). The results of this study showed that cultivars varied in the expression of a large number of gliadin and glutenin proteins. These variations, induced by genome and environment, may be significant for pasta quality and may vary for the same cultivar over years. PCA applied to 2DE data of gliadin and glutenin fractions showed that Wollaroi, Ocotillo, and Colosseo, having the highest protein expression and the highest number of spots for both the above fractions, would be the most suitable for pasta making. Further studies dealing with the identification of the above proteins may allow for better determination of the effect of the protein expression on pasta quality.

Durum wheat quality is also affected by starch (*48*). Functional attributes of starch are related to interactions between starch and water as influenced by temperature (e.g., gelatinization, pasting, gelation, and retrogradation) (*49*). The ratio amylose/amylopectin determines the physicochemical properties of starch and, thereby, the end-use of the durum wheat cultivar. Significant ($p < 0.05$) variations (61.0−73.2%) for total starch were found between the 10 semolina cultivars. The results of this study agreed with those previously reported (*11*). The concentration of amylose also varied between cultivars (13.0−33.6%). Amylose concentration appears to be under genetic control and varies over a limited range (16−28%) within durum wheat cultivars (*50*−*52*). Starch damage is the logical andàà inevitable consequence of all wheat milling processes. A narrow range of damaged starch was found for the 10 cultivars (2.8−3.9%). High levels of damaged starch increase the water penetration inside the dough and cause higher loss during cooking. These features give sticky texture, very bad mouth feel quality, and browning of the pasta color (*53*).

Overall, semolina with high SP is positively related to the softness of Japanese white salted noodles and negatively related to the firmness of yellow alkaline noodles (*33*). In the interspaces between starch granules, protein coagulation and interaction lead to the formation of a continuous and strengthened network, which traps starch, while starch itself, through swelling and gelatinizing, occludes these interspaces. Therefore, the faster the starch swells and its spherulites disperse during pasta cooking, the slower the protein−protein interaction occurs and the weaker protein network will be present in the resulting pasta (*54, 55*). The low permeability of the dough decreases the starch swelling capacity and renders starch more rigid and less soluble (*56*). As shown by other authors (*53, 56*), all durum wheat semolina cultivars of this study showed low values of SP.

RVA pasting properties of the 10 durum wheat semolina cultivars markedly varied. Overall, a negative relationship between amylose, PV, and SP was always found, but the 10 cultivars differed (*57*). SP and PV increased in reconstituted semolina with values of amylose ranging from 22.9 to 0.7%. High values of SP and PV negatively influence the cooking quality (*11, 58*). Cooking quality is separated into pasta texture after cooking and surface condition, which determine stickiness and degree of smoothness of pasta. These two features are relatively independent of each other (*4*). Although cooked faster, spaghetti made with semolina having a low concentration of amylose were too soft and did not resist overcooking (*59*). On the basis of starch characteristics, Wollaroi, Ocotillo, Duilio, Simeto, Ciccio, and Svevo cultivars should have higher performance during pasta making than the other cultivars (*58*−*60*).

Undoubtedly, the development of more sensitive and reliable techniques for helping producers of pasta to select durum wheat semolina cultivars is urgently needed. As stated by Skylas et al. (*41*), proteomics approaches using 2DE could fill the gap between gene expression and grain composition, as the part of "grain chain" events from breeding through agronomy and, finally, to processing and pasta quality. Because the search for protein markers is clearly a major objective for proteomic analyses, further work will consider the correlation between 2DE protein fingerprinting and pasta quality. Besides, the gluten network should be strong and elastic during cooking to prevent its destruction by swelling of starch granules. On the basis of the results of this study, an integrate approach between 2DE fingerprinting and concentration of amylose could be useful for selecting suitable durum wheat semolina cultivars for pasta making.

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